KARYOTYPE ANALYSIS OF THE RED SWAMP CRAYFISH, *PROCAMBARUS CLARKII*, BY CELL CULTURE

Karyological analysis of the red swamp crayfish, *Procambarus clarkii*, was examined using a cell culturing technique.

The specimens used for the present study were collected from a rice field near Mishima City, Shizuoka Prefecture, Japan.

The procedures of the cell culture and chromosome preparations were as follows. The bodies of the specimens were sterilized with 70% ethanol before the tissues were taken out. The antennal and genital glands were dissected out aseptically and cut into 1–2 mm pieces. The pieces of tissue were immersed in calcium- and magnesium- free Hank's solution for 20 minutes, and then in collagenase solution for 1–3 hours. The dissociated cells and small clumps of cells were collected by centrifuge (1,000 rpm for 5 minutes) and then washed in PC culture medium (Table 1). The collected cells and small clumps of cells were put into plastic tissue culturing petri dishes with new PC medium at 26°C. After the epithelial and fibroblastic cells increased sufficiently (3–7 days), colchicine (0.5 µg/ml) was added to the medium for 6–15 hours.

TABLE 1. Composition of the culture medium (PC).

Amino Acids	(mg/)	Salts	(g/l)
L-Arginine.HCl	70.0	NaCl	14.85
L-Aspartic acid	30.0	KCl	0.6834
L-Asparigine,H_2O	34.0	$MgSO_47H_2O$	0.9033
L-Aranin	25.0	$MgC1_26H_2O$	0.7451
β-Aranin	25.0	$CaC1_22H_2O$	2.2455
L-Cystin	20.0		
L-Glutamic acid	67.0		
L-Glutamin	100.0	Sugars	(g/l)
Clycine	50.0	Clucose	0.360
L-Histidine	22.0	Fructose	0.900
L-Isoleucine	20.0	Saccharose	0.684
L-Leucine	60.0		
L-Lysin.HCl	70.0		
L-Metionin	15.0	Buffer	(g/l)
L-Proline	40.0	HEPES	3.5745
L-Phenilalanin	25.0		
DL-Serin	25.0		
L-Tyrosin	40.0	Antibiotics	
L-Tryptophane	10.0	Gentamycin	100ug/ml
L-Threonine	30.0		
L-Valine	25.0	Serum	
L-Cystein.HCl	0.1	10% Fetal Bovin Serum	
L-Hydroxyproline	10.0		

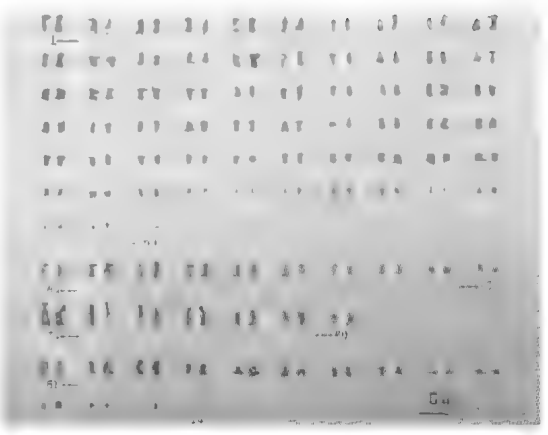

FIG. 1. Karyotype of the red swamp crayfish, *Procambarus clarkii*.

The increased cells were removed by trypsin treatment and treated with hypotonic solution (0.01 M KCl) for 6–12 minutes. The cells were fixed in fresh ethanol and acetic acid solution (3:1). The chromosome preparation was modified from our routine air-drying method for Crustacean chromosome preparations (Murofushi and Deguchi, 1983).

The diploid chromosome number of *P. clarkii* was 188 (2n) with considerable variation in size as was found by Murofushi *et al.* (1984). The chromosomes were classed as 63 pairs of meta-, 10 pairs of submeta-, 7 pairs of subtelo- and 14 pairs of acrocentrics (Fig. 1). The metacentric chromosome numbers formed 67%, and others were 15% in acro-, 10% in submeta- and 8% in subtelocentrics. The culture method provided more cells in mitotic metaphase than direct treatment with colchicine. The chromosomes were also more distinct.

Literature Cited

Murofushi, M and Deguchi, Y. 1983. A method for obtaining metaphase chromosomes from large shrimp-like crustaceans. Report of the Mishima Research Institute of Sciences for Living, Nihon University 6: 31–34.

Murofushi, M., Deguchi, Y and Yosida, T.H. 1984. Karyological study of the red swamp crayfish and the Japanese lobster by air-drying method. Proceeding of Japan Academy 60, Ser. B: 306–309.

M. Murofushi, Junior College at Mishima, Nihon University, Mishima, 411 Japan.

T. Nakatsubo and Y. Deguchi, Department of Fisheries, Nihon University, Setagaya, 154 Japan.